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Sequence diversity in the surface-exposed amino-terminal region of the coat proteins of seven strains of sugarcane mosaic virus correlates with their host range

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Summary. The N-terminal region of the coat proteins of five strains (Isis, Brisbane, Sabi, Bundaberg, and BC) of sugarcane mosaic virus (SCMV) isolated from four different plant species (sugarcane, sabi grass, wild sorghum, and blue couch grass) have been compared with the previously published data for SCMV-SC and SCMV-MDB, isolated from sugarcane and maize, respectively. The region, beginning at residue 11 and ending 16 residues beyond the second trypsin cleavage site of the coat protein, varied in size from 68 amino acid residues (Bundaberg) to 115 residues (BC) and contained repeat sequence motifs. Comparisons of the sequence identity and the nature of the repeats in the seven sequences showed that there were five different sequence patterns. These could be grouped further into three subsets which appeared to correlate with the host range of the strains. SCMV-Brisbane, SC, and Isis, isolated from sugarcane, showed almost identical sequence patterns and formed one subset. The other four strains had different sequence patterns and could be grouped further into a Sabi and Bundaberg subset (isolated from sabi grass), and a BC and MDB subset.

Introduction

Sugarcane mosaic virus (SCMV) is a definitive member of the genus *Potyvirus* in the family *Potyviridae* [2, 17, 22]. It occurs worldwide and infects maize, sorghum, sugarcane, and various grasses in the family *Gramineae*. Traditionally, isolates originating in sugarcane were designated SCMV [1] and those originating in maize were considered maize dwarf mosaic virus (MDMV) [10]. However, SCMV and MDMV appear to share many common properties and MDMV has been considered to be a strain of SCMV [14].

Based on the cross-reactivity of affinity-purified polyclonal antibodies di-

rected towards the surface-located, virus-specific N-termini of the coat proteins, 17 SCMV and MDMV strains from Australia and the U.S.A. were classified into four distinct potyviruses, namely Johnsongrass mosaic virus (JGMV), MDMV, sorghum mosaic virus (SrMV), and SCMV [16]. This classification was confirmed on the basis of several characters including: reactions of the strains on differential sorghum and oat cultivars [21]; cell-free translation of RNAs [3]; morphology and serology of cytoplasmic inclusions [7, 9]; amino acid sequences and peptide profiling of coat proteins [5, 8, 12, 13], and 3′ non-coding nucleotide sequences and molecular hybridization with probes corresponding to the 3′ non-coding regions of RNA [5, 6].

According to this new classification, the Australian SCMV strains BC, Brisbane, Bundaberg, Isis, Sabi, and SC, the American SCMV strains A, B, D, and E, and the B strain of MDMV (now referred to as SCMV-MDB), are accepted as strains of SCMV [17]. All of these strains were isolated from sugarcane except BC, Bundaberg, MDB, and Sabi. SCMV-BC was isolated from Queensland blue couch grass (*Digitaria didactyla*), SCMV-Bundaberg from wild sorghum (*Sorghum verticilliflorum*), SCMV-MDB from maize (*Zea mays*), and SCMV-Sabi from sabi grass (*Urochloa mosambicensis*) [18–20].

Recent comparisons of the deduced amino acid sequences of the coat protein genes of MDB and SC showed that they have much sequence identity and are better described as strains of the one potyvirus except that they have a region of unexpected diversity starting at residue 28 in the N-terminal region of their coat proteins. This divergent region in SC was smaller (44 residues) than the equivalent region in MDB (59 residues) and shared only 22% identity with the MDB sequence, compared with 92% identity for the remainder of the coat protein sequence [5]. The divergent regions in the coat proteins of both strains contained repeat sequences generated by partial gene duplication. In SC, the decapeptide comprising residues 18 to 27, was repeated exactly at residues 31 to 40. In MDB, the 17 residue peptide (residues 33–49) was duplicated at residues 50–66, but with some sequence changes. There was also a smaller exact duplication of residues 34–39 at 68–73 [5].

Coat proteins of plant viruses have been shown to be involved in several biological functions, including infectivity, virus transport, cross-protection, host range, symptom severity, and aphid transmission [11]. As SC and MDB originated in two different plant species, sugarcane and maize, respectively, it was of interest to determine whether SCMV strains with different host origins had different sequence motifs in the divergent region of the coat protein. In the work we report here, we have determined the nucleotide sequences of the divergent region of the coat protein gene from five more Australian SCMV strains originating in sugarcane or three other plant species, and we have examined the relationships between the derived protein sequences and the previously reported data for SC and MDB.

Materials and methods

Viruses and RNA isolation

The SCMV strains BC, Brisbane, Bundaberg, Isis, and Sabi were maintained in their perennial hosts [18, 19]. For the isolation of RNA, the strains were multiplied in sweet corn (*Zea mays*), cv Iochief, in an insect-proof glasshouse at 20–24 °C.

Total RNA was isolated from approximately 25 g of infected plant tissue using the extraction buffer and methods described previously [4]. The RNA was resuspended in 5 ml of water, precipitated with sodium acetate and ethanol, and the suspension was stored at − 20 °C. The concentration of plant RNA in this suspension was approximately 1 µg/µl.

cDNA synthesis, PCR amplification, and cloning

Aliquots (2 µg) of total RNA from infected plant tissue were used as templates for the production of oligo(dT)-primed cDNA using the Riboclone system (Promega). After completion of the synthesis, the reaction mixture (5 µl) was diluted to 20 µl with a buffer containing 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA, denatured by boiling, and then snap-frozen. For the polymerase chain reaction (PCR), 1 µl of the cDNA solution was amplified in a 100 µl reaction containing 25 nmoles of each primer (see below), 5 nmoles of each dNTP, 100 nmoles $MgCl_2$ and buffer supplied with the Pfu-DNA polymerase (Stratagene). Before adding 2.5 units of enzyme, the mixture was heated to 95 °C for 5 min and the primers were then annealed at 50 °C for 5 min. The mixture was then kept at 75 °C for 5 min before proceeding with thirty cycles of 95 °C, 50 °C, and 75 °C for 1 min at each temperature.

The mixed synthetic oligonucleotides used for the amplifications were based on sequences previously determined for SC and MDB, and spanned nucleotides coding for amino acids 2–10 for the 5′ primer, and residues 94–102 and 109–117 of SC and MDB coat proteins, respectively, for the 3′ primer [5]. The primers used were:

5′ Primer: 5′ GAACAGTCGATGCAGGCGCTCAAGG 3′
T T G T A

3′ Primer: 5′ GGTAAGCGCATTTTCTTTGACATGGC 3′
C C C

PCR products were examined by agarose gel electrophoresis, separated from unincorporated primers using Primerase columns (Stratagene), kinased and directly ligated to Sma I-digested and dephosphorylated M13 mp19 DNA.

DNA sequencing and computer analysis of DNA sequence data

Single-stranded DNA was prepared from recombinant clones and was sequenced using the AmpliTaq kit (Perkin Elmer) as instructed by the manufacturers. At least three sequences in each orientation were determined for each strain.

The Repeat program of the Sequence Analysis Software Package of the Genetics Computer Group, Version 6.0, was used to analyse repeat motifs of the nucleotide sequences. The program was run using a window of 10, a stringency of 8, and a range matching the length of insert. Major repeat motifs comprised 30 or more nucleotides.

Results and discussion

Amplification of the nucleotide sequences coding for the SCMV coat protein N-terminal variable regions

The primers used for amplification of the RNA coding for the coat protein N-terminal region were designed on the basis of the SC and MDB sequences

determined previously [5] and were chosen to yield PCR products which spanned the variable region. The 5′ primer began four nucleotides downstream of the start of the coat protein gene, and of the 25 nucleotides of the primer, 20 were identical in SC and MDB. The 3′ primer began 60 nucleotides downstream from where the variable regions of SC and MDB end. Twenty-three of the 26 nucleotides were identical.

cDNA of BC, Brisbane, Bundaberg, Isis, and Sabi as well as of SC cDNA, as a control [5] were amplified. Agarose gel electrophoresis of the PCR products showed a single discrete DNA band for each strain ranging in size from approximately 300 to 450 bp, with a similar yield for each sample (data not shown).

Sequences of the amplified gene products

Figure 1 shows the derived amino acid sequences of the variable N-terminal regions of the coat proteins of Isis, Brisbane, Sabi, Bundaberg, and BC compared to the previously determined data for SC and MDB [5]. The sequences started at the position equivalent to residue 11 in the coat proteins of SC and MDB and ended in the core region, 16 residues past the second trypsin cleavage site [15]. The sequences varied in length from 68 amino acids in Bundaberg to 115 amino acid residues in BC, with only two of the coat proteins (SC and Isis) having the same number of residues (83 amino acids) in this region (Fig. 1).

No sequence heterogeneity was observed among the sets of six clones of Isis or Brisbane and the sequence for the single SC clone examined matched the reported SC sequence [5]. There was limited sequence heterogeneity in the BC,

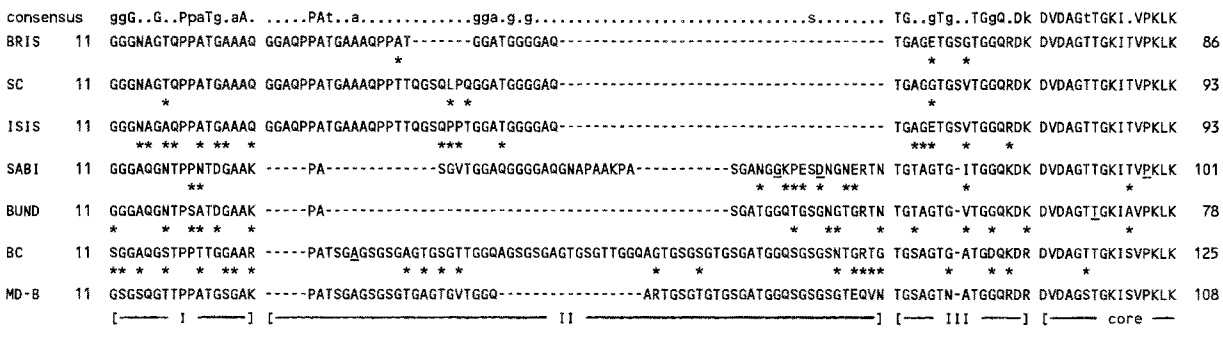

Fig. 1. The derived amino acid sequences of the N-terminal regions of five SCMV strains compared to the previously published data for SC and MDB [5]. The sequences have been aligned to show maximum identity, and the differences between adjacent pairs of sequences are denoted by asterisks. The four domains (*I–III* and *core*) have been separated by a space. The sequences begin at residue 11 and differ in size as shown. The DNA from six PCR-derived clones for each virus was sequenced. Minor differences (indicated by underlined amino acids) were observed in three clones of Sabi (Gly_{58} to Asp, Asp_{63} to Gly in all three clones, one of which also had the change Pro_{98} to Ser); one clone of Bundaberg (Thr_{69} to Lys); and one clone of BC (Ala_{33} to Ser). The consensus sequence shows those residues found in all seven SCMV strains (upper case) and those residues found in 4–6 of the sequences (lower case)

Bundaberg, and Sabi sequences (see legend to Fig. 1). A single change, Ala_{33} to Ser, was observed in one of the six clones of BC with the other five clones having the sequence shown in Fig. 1. A single change (Thr_{69} to Lys) was also observed in one of the six clones of Bundaberg, but this change was seven residues into the core region (Fig. 1). Sabi showed the greatest heterogeneity with only three of the six clones analysed having the sequence shown in Fig. 1. Two other clones were identical and had two changes (Gly_{58} to Asp and Asp_{63} to Gly) in the variable N-terminal region of their coat proteins. A third clone had these same two changes as well as a third change (Pro_{98} to Ser) in the coat protein core region (Fig. 1). The Gly residue at position 63 in these Sabi clones matched the Gly found at the corresponding position in the sequences of Bundaberg, BC, and MDB (Fig. 1). Although these differences may be due to misincorporation of nucleotides by the polymerase, the use of Pfu-DNA polymerase, an enzyme with proofreading activity, and the lack of any sequence heterogeneity in the other clone sets, suggested that the few differences observed reflected true diversity in the population of viral RNA molecules.

The sequences in Fig. 1 were arranged to highlight identities between strains. Four domains (I, II, III, and core) were recognized on the basis of the degree of identity in these regions. Domain I followed the sequence primed by the mixed oligonucleotides and spanned amino acid residues 11 to 27. As shown by the consensus sequence, there was much identity across the seven strains in this region. Of the 17 residues, 5 were identical across all strains, whereas 6 other positions were identical in at least 5 of the strains. The second region (domain II), represented the true hypervariable domain in the N-terminal region of the coat proteins of these strains. It varied in sequence across the seven SCMV strains with some strains (e.g., SC, Isis, and Brisbane) more closely related than the others (Fig. 1). Domain II also varied considerably in length from 21 residues in Bundaberg; 28 residues in Brisbane; 35 residues in SC and Isis; 44 residues in Sabi; 51 residues in MDB; to 68 residues in BC.

The hypervariable region was originally reported to be 44 residues long (residues 27–70) in SC and 59 residues long (amino acids 27–85) in MDB [5]. An analysis of the current, larger set of sequences allowed this hypervariable region to be redefined. It is nine (SC) and eight (MDB) residues shorter than previously suggested because of the high sequence identity in all seven strains in the region denoted as domain III (Fig. 1). Domain III contained 15 or 16 amino acid residues including the trypsin cleavage site, and was quite conserved with almost half of the residues identical in all strains (Fig. 1). The final amplified domain corresponded to the start of the coat protein core region [15] and was almost identical across all of the SCMV strains (Fig. 1), as expected for strains of the one potyvirus [22, 24].

Repeat motifs, first observed for SC and MDB [5], were also found in the new sequences. These motifs are shown in Fig. 2 and can be used along with the identity data in Fig. 1 to establish relationships between the seven strains of SCMV.

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          [—— I/II ——] [———————— II ————————] [—— III ——] [—— core —
BRIS  11  GGGNAGTQPPATGAAAQ
      28      GGAQPPATGAAAQ PPAT-------GGATGGGGAQ---------- TGAGETGSGTGGQRDK DVDAGTTGKITVPKLK  86

SC    11  GGGNAGTQPPATGAAAQ                                                                 27
      28      GGAQPPATGAAAQ PPTIQGSQLPQGGATGGGGAQ---------- TGAGGTGSVTGGQRDK DVDAGTTGKITVPKLK  93

ISIS  11  GGGNAGAQPPATGAAAQ                                                                 27
      28      GGAQPPATGAAAQ PPTIQGSQPPTGGATGGGGAQ---------- TGAGETGSVTGGQRDK DVDAGTTGKITVPKLK  93

SABI  11  GGGAQGNTPPNTDGAAK PA------------SGVTGGAQG                                        38
      39  GGGAQGNAP-----AAK PA------------SGANGGK----------PESDNGNERTN TGTAGTG-ITGGQKDK DVDAGTTGKITVPKLK 101

BUND  11  GGGAQGNTPSATDGAAK PA------------SGATGGQ----------TGSGNGTGRTN TGTAGTG-VTGGQKDK DVDAGTTGKIAVPKLK  78

BC    11  SGGAQGSTPPTTGGAAR PATSGAGSGSGAGTGSGTTGGQ                                        49
      50                    AGSGSGAGTGSGTTGGQ                                             66
      67                    AGTGSGSGTGSGATGGQ----------SGSGSNTGRTG TGSAGTG-ATGDQKDR DVDAGTTGKISVPKLK 125

MD-B  11  GSGSQGTTPPATGSGAK PATSGAGSGSGTGAGTGVTGGQ                                        49
      50                    ARTGSGTGTGSGATGGQ----------SGSGSGTEQVN TGSAGTN-ATGGQRDR DVDAGSTGKISVPKLK 108
          [—— I ——] [———————— II ————————] [—— III ——] [—— core —
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Fig. 2. Repeat sequences in the N-terminal regions of the coat proteins of seven strains of SCMV. The repeat sequences as well as additional, partial repeats are underlined. Gaps have been introduced to aid alignments of the different patterns

Patterns of sequence variation in seven strains of SCMV

The sequence data for the N-terminal regions of the coat proteins of the seven strains of SCMV revealed differences in the nature and extent of gene duplication in this region of the viral genome. Whereas all sequences were related to some extent, there appeared to be five patterns, with some of these patterns more closely related than others.

The first pattern was shown by Brisbane, SC, and Isis, which all had a complete repeat of domain I residues ^{16}GAQPPATGAAAQ^{27} (underlined in Fig. 2). These three strains also showed different degrees of a third, partial repeat of this sequence and were also almost identical for the rest of their N-terminal sequences. As shown in the pairwise comparisons in Fig. 1, there was only one sequence difference in domain I (at residue 17 in Isis); two sequence differences in domain III (at residues 66 in SC and 70 in Brisbane) and no sequence differences in the first 16 residues of the core. The Brisbane strain had a seven residue deletion (residues 45–51) and a change at residue 43 when compared with SC, while the Isis and SC strains differed at only two positions (residues 49 and 51) in domain II.

The second pattern was shown by Sabi which had an almost perfect repeat of residues 11–19 and 25–35 (Fig. 2). As shown in Fig. 1, the sequence ^{34}GGAQGGGGAQ^{43} in domain II of Sabi had counterparts in domain II of the SC/Isis/Brisbane subset (Fig. 1), whereas the overlapping sequence ^{30}SGvTGG^{35} had counterparts in Bundaberg, BC, and MDB (Fig. 2). The

domain I sequence in Sabi differed from those of Brisbane, Isis, and SC at eight positions, and from those of BC and MDB at five and seven positions, respectively, but was almost identical to that of Bundaberg (Fig. 1).

The third pattern was shown by the Bundaberg strain which had no significant repeats other than the TGGQ sequence at residues 33–36 in domain II and 56–59 in domain III (Fig. 2). This sequence motif was present seven residues upstream of the core in all of the strains except BC, where the second Gly was replaced by Asp. Overall, the Bundaberg sequence showed very high identity with the domain I and III sequences of Sabi (with only two differences in the former and one difference in the latter), and with the consensus sequence of the Sabi strain repeat (Fig. 2). This suggested that the Sabi and Bundaberg patterns may form a second subset among these SCMV strains. However, there were five differences between Bundaberg and Sabi in the last eleven residues of domain II (Fig. 1) and the domain II sequence of Bundaberg (20 amino acids) could be aligned as well with that of BC (only four differences) although it was much shorter (Figs. 1 and 2).

The fourth pattern was shown by BC which had one perfect repeat of the sequence, ^{33}AGSGSGAGTGSGTTGGQ^{49} at residues 50–66, followed immediately by a second repeat where only three of the 17 residues were different (Fig. 2). This was followed by a third sequence, ^{84}SGSGsnTGrtgTG^{96}, where the residues shown in uppercase matched the original repeating unit. The first two copies of the 17 residue repeat element in BC showed 98% identity at the nucleotide level (data not shown).

The fifth pattern was shown by MDB. Its domain II sequence was very similar to that of BC but had only two copies of the 17 residue repeat and a slightly different form (^{67}SGSGsGTeqvnTG^{79}) of the partial repeat seen in the BC strain (Fig. 2). The striking similarity between the repeating units in domain II suggested that BC and MDB could form a third subset among the SCMV strains. This was supported further by the additional, partial duplications of the MDB domain I sequence ^{20}PATGSGA^{26} at the start of domain II in both strains. However, the domain I sequences of BC and MDB were the most different of all the SCMV strains examined, differing at eight of the 17 positions (Fig. 1). There were also five differences at the end of domain II.

Correlation with host range

The data summarized in Table 1 suggested that the sequence patterns correlated with biological properties such as host range. The Australian SCMV strains (SC, Isis, and Brisbane) formed one subset as they had almost identical N-termini and very similar biological properties. They infect sugarcane, can also infect maize and wild sorghum, but not blue couch grass and sabi grass, the perennial hosts of BC and Sabi [18, 19]. It will be interesting to determine by sequence and host range analyses, whether the American SCMV strains (A, B, D, and E), isolated from sugarcane, also fall into the SC/Isis/Brisbane subgroup.

The SCMV strains which can be isolated from blue couch grass or sabi grass

Table 1. Host range correlations of SCMV strains[a]

Strain	Sugarcane	Maize	Infectivity		
			wild sorghum	sabi grass	blue couch grass
SC	yes	yes	yes	no	no
Isis	yes	yes	yes	no	no
Brisbane	yes	yes	yes	no	no
Sabi	no	yes	yes	yes	no
Bundaberg	no	yes	yes	yes	no
BC	no	yes	yes	no	yes
MDB	no	yes	ND	ND	ND

[a] Data based on previous studies [18–20] and D. S. Teakle (unpubl. obs.)
ND Not determined

do not infect sugarcane [18–20]. Furthermore, BC and Sabi appear to belong to distinct subsets as they do not infect each other's perennial hosts [19].

The possible subgrouping of Bundaberg with Sabi is supported by the fact that Bundaberg can infect sabi grass (Table 1), and by the observation that Bundaberg and Sabi are the only strains so far able to infect a dicotyledonous host, French bean (*Phaseolus vulgaris*) where they cause latent infections of the inoculated French bean leaves [18, 19]. These two strains are also very similar in their effects on other hosts [18, 19]. The possible alternative subgrouping of Bundaberg with BC is less favoured since BC is unable to infect sabi grass, a host for Bundaberg [19].

Finally, the almost identical nature of the 17 residue repeat in the hypervariable region of the coat proteins of BC and MDB (Fig. 2) suggested that these strains form a third subset. This subgrouping is supported by cross-protection data (B. Krstic and M. Tosic, pers. comm.), which show that MDB infection can protect sweet corn seedlings from subsequent infection with BC, but not Sabi or SC. This cross-protection is unidirectional and is not observed when BC is the first inoculum and MDB is the challenge virus. No cross-protection is observed between MDB and SC, or MDB and Sabi in either combination of inoculation and challenge. The host range similarity of BC and MDB is incomplete as MDB is not present in Australia and has not been inoculated to blue couch grass or sabi grass (Table 1).

It is interesting to note that MDMV and SrMV, two other potyviruses infecting sugarcane, maize and sorghum [16, 17], also have sequence duplication in the N-terminal region of their coat proteins, but the repeated sequences are totally unrelated to each other and to any of the repeat sequence motifs described here [8]. To investigate whether repeated sequence elements in the N-termini of coat proteins are restricted to those potyviruses that infect *Gramineae*, the N-terminal regions of all the available potyvirus coat protein sequences have

been examined and evidence found for partial gene duplication in most of the coat proteins that are 287 amino acid residues or longer [23, 25]. These observations suggested that partial gene duplication has been a frequent mechanism for generating diversity in the virus-specific, N-terminal region of the coat protein of potyviruses and that the capacity to change the coat protein without affecting its ability to form virus particles may have been an important factor in the evolution of new species of potyviruses with new host specificities.

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